

The Fermentation of Glucosides by Bacteria of the Typhoid-coli Group and the Acquisition of New Fermenting Powers by Bacillus dysenteriae and other Micro-organisms. Preliminary Communication.

By F. W. TWORT, M.R.C.S., L.R.C.P.

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(From the Bacteriological Laboratory, London Hospital, E.)

For a number of years bacteriologists have largely relied upon certain fermentation reactions of sugars and alcohols for the differentiation of various micro-organisms which may otherwise be almost indistinguishable. This applies more especially to the case of bacteria occurring in the so-called "typhoid-coli group," although recently this method of differentiation has also been extended by Mervyn Gordon (1), Andrewes, Horder (2), and others, to the group of micro-organisms described under the general name of Streptococcus. As fresh fermentible substances have been introduced as tests, the number of different species occurring in such micro-organismal groups has become very large. Mervyn Gordon, in his studies on Streptococcus and Staphylococcus, introduced tests on certain sugars and upon such glucosides as coniferin and salicin, and he considered that these reactions were of help in differentiating different Streptococci from each other. MacConkey (3) has also published extended observations on the capability of certain lactose fermenting faecal bacteria to attack sugars like saccharose and dulcitate, and from the reactions obtained he was led to subdivide these lactose fermenters into four groups as follows:—

- (1) A group represented by *Bacillus acidi lactici*, Hueppe, capable of fermenting lactose, but without action on dulcitate or saccharose.
- (2) A group, the typical representative of which is *Bacterium coli*, Escherich. In this case lactose and dulcitate are fermented, but not saccharose.
- (3) A group of the types *Bacillus neapolitanus*, Emmerich, and *Bacillus pneumoniae*, Friedländer, containing organisms which ferment lactose, saccharose, and dulcitate.
- (4) A group containing micro-organisms capable of fermenting lactose and saccharose, but not dulcitate. In this group were placed *Bacillus lactis aerogenes*, Escherich, *Bacillus capsulatus*, Pfeiffer, and *Bacillus cloacae*, Jordan.

On utilising these sugar tests, MacConkey was able to differentiate such closely allied bacteria as *B. coli*, Escherich, *B. acidi lactici*, Hueppe, *B. lactis aerogenes*, Escherich, and *B. pneumoniæ*, Friedländer.

It was with a view to obtaining other fermentation tests which might be of service in separating organisms still remaining undifferentiated that the following experiments with glucosides were undertaken.

In carrying out these tests, an ordinary peptone water medium containing 2 per cent. of the glucoside to be tested was used. Such a medium was distributed in a number of test tubes, each of which contained a small inverted glass tube sealed at the upper end after the method recommended by Durham. In one series of experiments litmus solution was purposely left out of the medium to see whether any colour change might be observable in the glucoside under examination. In another series 5 per cent. of Kahlbaum's litmus solution was added, the medium being otherwise identical with the above. All the glucosides used were obtained from Merck.

In the case of insoluble glucosides an amount was added to the peptone water which would represent a 2-per-cent. solution.

All those media containing glucosides which by their colour obscured the reaction of the litmus solution were repeatedly tested on litmus paper during the growth of the organism.

Forty-nine glucosides were tested with the following 18 species of bacteria belonging to the typhoid-coli group:—

B. fecalis alcaligenes, Petruschky.

B. dysenteriae, Kruse.

„ Flexner (Pseudo-dysentery IV of Firth).

B. typhosus, Eberth-Gaffky.

B. paratyphosus, Brion and Kayser.

„ Schottmüller.

B. enteritidis, Gaertner.

B. paracoli, Widal.

B. coli, Escherich.

B. pneumoniæ, Friedländer (MacConkey strain).

„ „ (laboratory strain).

„ „ (Král strain).

B. neapolitanus, Emmerich.

B. pyogenes fætidus, Passet.

B. acidi lactici, Hueppe.

B. lactis aerogenes, Escherich.

B. capsulatus, Pfeiffer.

B. cloacæ, Jordan.

At least six tubes of each glucoside medium were examined, the period of observation extending over four weeks.

Of the 49 glucosides examined, the following showed no reaction which could be ascribed to fermentation:—

Ononin.	Jalapin.	Convallarin.
Ericolin.	Scammonin.	Quabin.
Digitalein.	Colocynthin.	Scopolin.
Helleborein.	Plumierid.	Frangulic acid.
Cyclamin.	Absynthin.	Smilacin.
Apiin.	Quercitrin.	Condurangin.
Hederagluside.	Hesperidin.	Convolvulin.
Tannin.		

The glucoside plumierid gave a dark green coloration with *B. capsulatus* and to a less extent with *B. lactis aerogenes*, *B. cloacæ*, and *B. pneumoniæ*, but no real fermentation was observed.

The remaining 27 glucosides showing some degree of fermentation with one or more of the test micro-organisms were as follows:—

Euonymin green.	Periplocin.	Populin.
Euonymin brown.	Cathartinic acid.	Camellin.
Iridin.	Amygdalin.	Globularin.
Senegin.	Sapotoxin.	Cerberid.
Coniferin.	Saponin.	Baptisin.
Arbutin.	Bryonin.	Coronillin.
Salicin.	Convallamarin.	Gratiolin.
Syringin.	Digitalin.	Adonidin.
Quillajinic acid.	Strophanthin.	Phloridzin.

In addition to the 18 test bacteria mentioned above, experiments were also carried out in the glucoside media with 26 other members of the typhoid-coli group. The latter cultures were isolated in the London Hospital from cases mostly of cystitis and colitis.

The results obtained with the whole 44 bacteria are set forth in the accompanying table, the type cultures are distinguished by an asterisk.

The sign + indicates a positive reaction, the sign — indicates that there was no change in the glucoside under observation. Vacant spaces indicate that no tests were made, the quantities of glucoside available being insufficient. "A" is used to denote acid formation; "G" gas formation.

In the table it can be seen that the bacteria have been subdivided into seven sub-groups according to their fermentative action on glucose, lactose, dulcitol, and saccharose.

The last four of these sub-groups correspond to the four groups of MacConkey.

On looking at the table it is apparent—

1. That there is comparatively little difference between the fermenting capabilities of many of the lactose fermenters.

2. That two micro-organisms of a sub-group may differ in their ability to ferment glucosides, whereas micro-organisms in different sub-groups may present the same fermentative changes when tested on glucosides.

It will also be noted that the chief fermentations occur in the four sub-groups of lactose fermenters, but whereas the members of the true coli group give practically identical results when tested on glucosides, the results with members of the Friedländer group show greater variations, the chief of which is the inability of *B. neopolitanus* and Bacillus No. 41 to ferment salicin, arbutin, and syringin, which glucosides are easily fermented by *B. friedländer*. It must also be pointed out that the fermentation of many glucosides is neither marked nor constant.

Taking the individual glucosides in the table and reading from left to right, the following are the chief general characteristics of the fermentations:—

Brown euonymin.	Gas production very variable, often marked. Acid production seldom present, never marked.
Green euonymin.	Gas and acid formation never very marked.
Iridin and senegin.	Acid and gas extremely variable, often marked.
Coniferin, arbutin, salicin, syringin.	Usually show very distinct fermentation, both acid and gas being frequently very marked, occasionally slight.
Quillajinic acid.	Gas sometimes marked, acid always slight.
Populin.	Gas never excessive, acid production very variable.
Camellin, globularin, cerberid, periplocin, cathartinic acid.	Gas production always slight, acid never very pronounced.
Amygdalin.	
Sapotoxin.	Gas never formed, acid production variable and sometimes marked, especially with members of Capsulatus and Friedländer sub-groups.
Saponin.	Gas production always slight, acid variable, but most pronounced, with Capsulatus and Friedländer sub-groups.
Bryonin and convallamarin.	Gas very rarely present, acid usually slight.
	Acid and gas never very pronounced and very inconstant.

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No.		Euonymin (brown).		Euonymin (green).		Iridin.		Senegin.		Coniferin.		Arbutin.		Salicin.	
		A	G	A	G	A	G	A	G	A	G	A	G	A	G
1*	<i>Bacillus faecalis alcaligenes</i> , Petruschky (Conradi strain)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2*	<i>Bacillus dysenteriae</i> , Kruse (Král strain)	—	—	—	—	+	—	+	—	—	—	—	—	—	—
3*	<i>Bacillus dysenteriae</i> , Flexner (Firth strain)	—	—	—	—	+	—	+	—	—	—	—	—	—	—
4*	<i>Bacillus typhosus</i> , Eberth (Danish strain)	—	—	—	—	+	—	+	—	—	—	—	—	—	—
5*	<i>Bacillus paratyphosus</i> , Schottmüller (Král strain)	—	+	—	+	+	+	+	+	—	—	—	—	—	—
6*	<i>Bacillus enteritidis</i> , Gaertner (Král strain)	—	+	—	+	+	—	+	—	—	—	—	—	—	—
7*	<i>Bacillus paratyphosus</i> , Brion and Kayser (Král strain)	—	+	—	+	+	—	+	—	—	—	—	—	—	—
8*	<i>Bacillus paracoli</i> , Widál (Král strain)	—	+	—	+	+	—	+	+	—	—	—	—	—	—
9	<i>Bacillus</i> from urine—cystitis	—	—	—	—	+	—	—	—	—	—	—	—	—	—
10	<i>Bacillus</i> from urine—cystitis	—	+	—	—	+	—	—	—	—	—	—	—	—	—
11*	<i>Bacillus acidi lactici</i> , Hueppe (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+
12	<i>Bacillus</i> from stool—colitis	—	+	+	—	+	+	+	+	—	—	+	+	+	+
13	<i>Bacillus</i> from stool—colitis	—	+	+	+	+	+	+	+	—	—	+	+	+	—
14	<i>Bacillus</i> from urine—cystitis	—	+	+	+	+	+	+	+	—	—	+	+	+	+
15	<i>Bacillus</i> from urine—cystitis	—	+	+	+	+	+	+	—	—	—	+	+	+	+
16	<i>Bacillus</i> from stool—colitis	—	+	+	+	+	+	+	+	—	—	+	+	+	+
17*	<i>Bacillus coli</i> , Escherich (MacConkey strain)	—	+	+	—	+	+	+	+	+	+	+	+	+	+
18	<i>Bacillus</i> from stool—colitis	—	+	+	+	+	+	+	+	—	—	+	+	+	+
19	<i>Bacillus</i> from stool—colitis	—	+	+	—	+	—	+	+	—	—	+	+	+	+
20	<i>Bacillus</i> from stool—colitis	—	+	+	+	+	+	+	+	—	—	+	+	+	+
21	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	—	—	—	+	+	+	+
22	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	+	—	—	+	+	+	+
23	<i>Bacillus</i> from urine—cystitis	—	+	+	—	+	+	+	+	—	—	+	+	+	+
24	<i>Bacillus</i> from urine—cystitis	—	+	+	—	+	+	+	—	—	—	+	+	+	+
25	<i>Bacillus</i> from urine—cystitis	—	+	+	+	+	+	+	—	—	—	+	+	+	+
26	<i>Bacillus</i> from stool—colitis	—	+	+	—	+	+	+	—	—	—	+	+	+	+
27	<i>Bacillus</i> from urine—cystitis	+	+	+	+	+	+	+	+	—	—	+	+	+	+
28	<i>Bacillus</i> from urine—cystitis	—	+	+	—	+	+	+	+	—	—	+	+	+	+
29*	<i>Bacillus pneumoniae</i> , Friedländer (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+
30	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	+	—	—	+	+	+	+
31	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	+	—	—	+	+	+	+
32	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	+	—	—	+	+	+	+
33	<i>Bacillus</i> from urine—cystitis	—	+	+	—	+	+	+	+	—	—	+	+	+	+
34*	<i>Bacillus pneumoniae</i> , Friedländer (laboratory strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+
35	<i>Bacillus</i> from sputum	+	+	+	—	+	+	+	+	—	—	+	—	+	+
36	<i>Bacillus</i> from stool—colitis	—	+	+	+	+	+	+	+	—	—	+	+	+	+
37*	<i>Bacillus pneumoniae</i> , Friedländer (Král strain)	—	+	+	—	+	+	+	—	—	—	+	—	+	+
38	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	+	—	—	+	—	+	—
39*	<i>Bacillus pyogenes fetidus</i> , Passet (MacConkey strain)	—	—	+	+	+	—	+	—	+	—	+	—	+	—
40*	<i>Bacillus neapolitanus</i> , Emmerich (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	—	—	—	—
41	<i>Bacillus</i> from stool—colitis	+	+	+	+	+	+	+	—	—	—	—	—	—	—
42*	<i>Bacillus capsulatus</i> , Pfeiffer (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+
43*	<i>Bacillus lactis aerogenes</i> , Escherich (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	+
44*	<i>Bacillus cloacae</i> , Jordan (MacConkey strain)	—	+	+	+	+	+	+	+	+	+	+	+	+	—

[illegible]

Digitalin and strophanthin. Gas always small, acid production often well marked.

Coronillin, gratiolin, baptisin, adonidin. } Acid and gas usually slight and inconstant.

Phloridzin. No gas production, acid formation slight.

Colour changes are observed in the case of certain glucosides, the most striking being the following.

Media containing *coniferin* often turn yellow or orange even although no apparent fermentation has taken place. Arbutin-containing media frequently turn brown when the cultures are old and alkaline. This is quite independent of any sign of fermentation.

Syringin media turn to a bright pink colour when fermented. In some instances, however, the colour is pale.

Amygdalin media turn lemon yellow in old alkaline cultures, showing no fermentative change.

Phloridzin media inoculated with *B. capsulatis*, *B. cloacæ*, *B. pneumoniæ*, *B. lactis aerogenes*, and Bacilli Nos. 30, 31, 32, 33, 38 and 41, show a bright brick red colour.

Plumierid becomes dark green in old alkaline cultures of *B. lactis aerogenes*, and the same may occur to a less extent with other micro-organisms.

The results quoted above seem to show that there is no sharp line of demarcation between the various sub-groups of lactose fermenting micro-organisms. It is true that nearly all the bacteria tested show slight individual characters, but they are so closely allied to each other that attempts to group them according to their lactose, dulcitol, and saccharose fermenting powers must be regarded as artificial.

It seems, therefore, probable that the separate micro-organisms in the various sub-groups are not to be regarded as distinct species, but as varieties or hybrids of one or more species. If this be so, one might expect them to be constantly varying, losing old characters and gaining new ones according to the conditions under which they are grown, and it was with the object of testing this hypothesis that further series of experiments were undertaken. In the first place attempts were made to hybridise such organisms as *B. coli* and *B. typhosus*, but owing to technical difficulties no conclusive results were obtained.

Attempts were then made to produce variations in the pure cultures of the 18 test types of micro-organisms, the method employed being to grow each micro-organism for a succession of generations in a fluid medium containing a sugar which it had failed to ferment, the object being to see

how far it could acquire a new fermenting property. After these experiments were practically finished, my attention was directed to a paper by Oscar Klotz (4), who had used the same method on one particular microbe of the coli group.

Klotz, however, only attempted to make his microbe regain characters which it had lost. In this he was successful after daily sub-cultures were made for about four generations, but it must be noted that his organism regained its fermentative powers even when grown on ordinary agar, although in this instance it was slower. The case with which Klotz was dealing was really one in which certain physiological functions were lost after the bacterium had passed through the body of an animal, these functions being regained in sub-cultures *in vitro*.

In the experiments which I have carried out the micro-organisms chosen had been grown in the laboratory for some years, their origin and properties being thoroughly well known. Many of them were obtained directly from Dr. MacConkey.

The media used in the experiments contained a sugar which the particular microbe is ordinarily unable to ferment. The sugar was added to ordinary salt peptone water to the extent of 2 per cent.

To ensure that the cultures were pure, they were first plated out, sub-cultures being obtained from single colonies. These sub-cultures were then passed through the usual sugar tests, being grown for four weeks at 37° C. Each culture so obtained and proved to be pure was transferred to a medium containing the sugar which at first it had failed to ferment. In such a medium it was allowed to grow for 14 days, when sub-cultures were again made to the same sugar medium and so on for successive generations.

All the sub-cultures were incubated for four weeks, the experiments being prolonged over months. The reason why the cultures were incubated for 14 days was to try to induce the microbe to attack the sugar after it had used up the other nutritive material in the medium. If division was still going on, it was assumed that any variety with a tendency to ferment the particular sugar would survive and multiply, since it could utilise the latter which the other members of the culture could not. In this way it might be expected that in successive generations a culture of increasing fermentative power would be obtained.

Such indeed proved to be the case; for several microbes were ultimately able to attack sugars which they, in accordance with universal bacteriological experience, at first failed to do. This change of function was slow, in most cases many successive generations being required before the new property was fully developed. Thus all the members of the para-typhoid sub-group

were ultimately able to ferment saccharose. *B. typhosus* also acquired the property of fermenting dulcite and lactose.

The dysentery bacilli of Kruse and Flexner were ultimately able to ferment saccharose within 24 hours. *B. acidi lactici*, after several generations, fermented saccharose, and thus fell into the same sub-group as *B. lactis aerogenes*. *B. dysenteriae* Kruse was also induced to ferment lactose, and so, fermenting glucose, saccharose, and lactose, came to be placed in the same sub-group with *B. lactis aerogenes*, and in this respect may be regarded as having fallen from the top of the typhoid-coli group to the bottom. Similar changes in the case of other micro-organisms ultimately led to the belief that division into distinct sub-groups separated by fermentation tests is impossible. The fermentation reactions are characterised by acid reactions, but are rarely accompanied by the production of gas. Special experiments were carried out with a typhoid bacillus which had acquired the power to ferment dulcite. When such a culture was plated out on agar, sub-cultures from single colonies retained the dulcite disruptive powers, although they were still capable of being agglutinated by a typhoid immune serum, thus proving that the fermentation was not due to any contaminating microbe. On inoculating the dulcite fermenting typhoid culture into a guinea-pig, sub-cultures were obtained showing the same reactions and these reactions were also maintained, even when the microbe was grown for several generations on ordinary peptone agar.

Conclusions.

1. A large number of glucosides may be fermented by many members of the typhoid-coli group of bacteria. The fermentations vary with the micro-organism tested, and the variations are as marked inside each sub-group of bacteria as between adjacent sub-groups.

2. The sugar-fermenting powers of an organism may be artificially changed by growing the said organism for a succession of generations in media containing a sugar which at the commencement of the experiment it was unable to ferment.

By this means a pathogenic organism may be altered until it gives fermentative reactions characteristic of a non-pathogenic member of its group. It is possible, indeed, that pathogenic organisms in the typhoid-coli group may so alter their characters that they become unrecognisable when growing for some time outside the body in soil, water, etc.

If this is so, it might partly account for the difficulty experienced in isolating *B. typhosus* from these situations.

It also seems possible that a non-pathogenic organism may lose its

fermenting powers and become pathogenic should it find a suitable medium such as the alimentary canal, and regain its old characters when outside the body. This is, however, only a suggestion which at present is in no way proved.

In view of the results obtained with the typhoid-coli group of organisms, it seems quite possible that other organisms may show similar changes, and that the fermentation tests worked out by Mervyn Gordon for the Streptococci may also be inconstant if the same means of experimentation are employed.

In conclusion, I have to thank the Royal Society for the Government grants which enabled me to purchase the glucosides used in this research, and also Dr. MacConkey for many of the typical cultures used.

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District	Name of the fishery	2019					2020					2021					2022					2023					2024				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
District 1	1. Fishery "Tugay-Fish" (District 1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2. Fishery "Tugay-Fish" (District 1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3. Fishery "Tugay-Fish" (District 1)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 2	4. Fishery "Tugay-Fish" (District 2)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5. Fishery "Tugay-Fish" (District 2)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6. Fishery "Tugay-Fish" (District 2)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 3	7. Fishery "Tugay-Fish" (District 3)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8. Fishery "Tugay-Fish" (District 3)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	9. Fishery "Tugay-Fish" (District 3)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 4	10. Fishery "Tugay-Fish" (District 4)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11. Fishery "Tugay-Fish" (District 4)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	12. Fishery "Tugay-Fish" (District 4)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 5	13. Fishery "Tugay-Fish" (District 5)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	14. Fishery "Tugay-Fish" (District 5)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	15. Fishery "Tugay-Fish" (District 5)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 6	16. Fishery "Tugay-Fish" (District 6)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	17. Fishery "Tugay-Fish" (District 6)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	18. Fishery "Tugay-Fish" (District 6)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 7	19. Fishery "Tugay-Fish" (District 7)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	20. Fishery "Tugay-Fish" (District 7)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	21. Fishery "Tugay-Fish" (District 7)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 8	22. Fishery "Tugay-Fish" (District 8)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	23. Fishery "Tugay-Fish" (District 8)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	24. Fishery "Tugay-Fish" (District 8)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 9	25. Fishery "Tugay-Fish" (District 9)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	26. Fishery "Tugay-Fish" (District 9)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	27. Fishery "Tugay-Fish" (District 9)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District 10	28. Fishery "Tugay-Fish" (District 10)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	29. Fishery "Tugay-Fish" (District 10)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	30. Fishery "Tugay-Fish" (District 10)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1